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Prospects for the gliding mechanism of *Mycoplasma mobile*

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Mycoplasma mobile forms gliding machinery at a cell pole and glides continuously in the direction of the cell pole at up to 4.5 μm per second on solid surfaces such as animal cells. This motility system is not related to those of any other bacteria or eukaryotes. *M. mobile* uses ATP energy to repeatedly catch, pull, and release sialylated oligosaccharides on host cells with its approximately 50-nm long legs. The gliding machinery is a large structure composed of huge surface proteins and internal jellyfish-like structure. This system may have developed from an accidental combination between an adhesin and a rotary ATPase, both of which are essential for the adhesive parasitic life of *Mycoplasmas*.

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Introduction

The class *Mollicutes*, which includes *Mycoplasma*, *Spiroplasma*, and *Acholeplasma*, is a class of parasitic or commensal bacteria featuring reduced genome sizes (560–2300 kbp) [1]. Phylogenetically, *Mollicutes* belong to the low-GC branch of Gram-positive bacteria, which also includes *Clostridium* and *Bacillus*. *Mollicute* species are classified into four subgroups, Hominis, Pneumoniae, Spiroplasma, and Phytoplasma [2,3]. Their cells lack a peptidoglycan layer and are covered with membrane-anchored proteins, including surface proteins responsible for adhesin and antigenic variation [4].

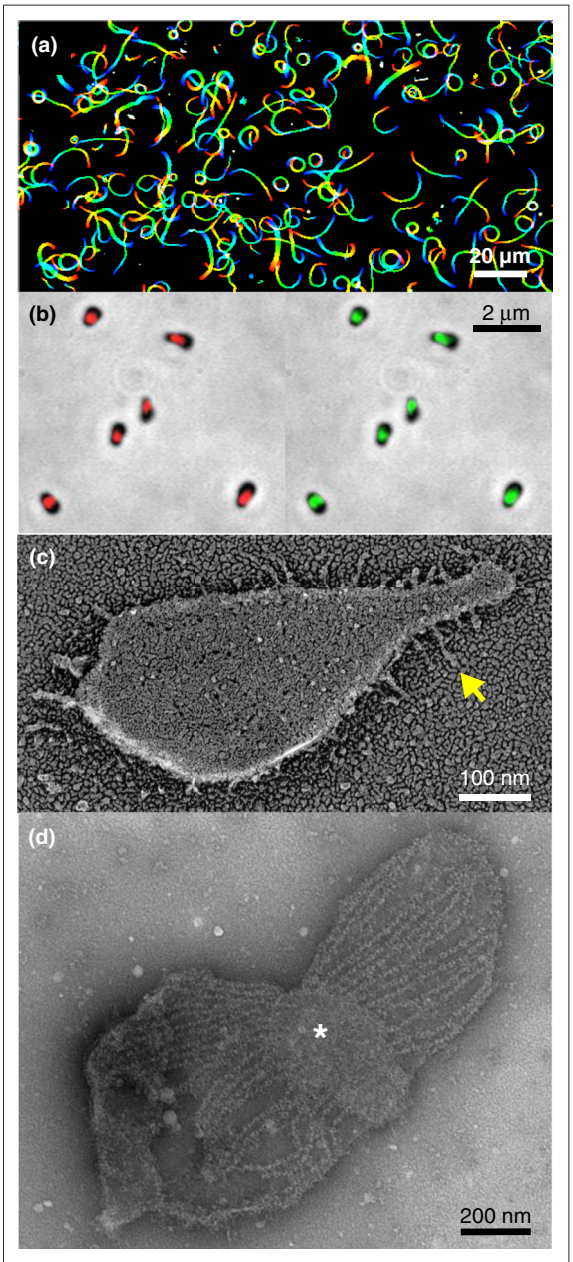
Certain *Mollicute* species glide on solid surfaces (Figure 1a) [5–8]. They form a membrane protrusion at a cell pole and glide in the direction of the protrusion, and this motility is thought to be involved in their parasitism. The gliding species belong to two subgroups, that is, the Hominis subgroup represented by *Mycoplasma mobile* and the Pneumoniae subgroup, which are phylogenetically distant from each other and do not share the proteins involved in gliding [6••,9,10]. Interestingly, *Spiroplasma* species 'swim' by another unique mechanism [11,12]. The motility systems of *Mollicutes* are not related to any other bacterial systems, such as flagella, pili, Gram-negative gliding or conventional motor proteins such as myosin that are involved in eukaryotic motility [9,10]. Therefore, *Mollicutes* move by three unrelated unique mechanisms (dozens of video files are available through our online video library, http://bunshi5.bio.nagoya-u.ac.jp/~mycmobile/video/).

M. mobile, a fish pathogen, is the fastest of the gliding species. It glides smoothly and continuously on glass at an average speed of 2.0–4.5 μm per second, or 3–7 times the length of the cell per second [13–15]. In this report, we overview the studies on *M. mobile* gliding published from 1997 to date, and we suggest an updated mechanism by incorporating recent information.

Surface structure

The proteins involved in gliding were identified through two lines of experiments, that is, the isolation of nonbinding mutants and the isolation of inhibitory antibodies [16–20]. The antibodies against the proteins missing in nonbinding and nongliding mutants inhibited binding and gliding. Three proteins coded tandemly on the genome essential for gliding localized at the base of the protrusion formed at a cell pole (Figure 1b). These proteins are named Gli123, Gli349, and Gli521, coded as ORFs of 1128, 3181, and 4728 amino acids have large molecular masses of 123, 349, and 521 kDa, respectively, and localize at a density of approximately 450 molecules per cell for each all around the neck part [20]. Considering the numbers and sizes of the proteins and the space constraints of the subcellular site, the complexes are probably to be aligned in a two-dimensional sheet (Figure 2a). Gli349 is essential for binding and gliding, and monoclonal antibodies against this protein detach the gliding cells from the solid surfaces [16,18,19,21]. The isolated molecule 95 nm long is shaped as an eighth note in written music, and shows binding activity to sialylated oligosaccharides (SOs), the scaffold for mycoplasma gliding (see below) [22–24]. Therefore, this protein has been assigned the role of 'leg' [16,18,19,21,22]. Gli521 is also essential for binding and gliding, and the monoclonal antibodies against this protein stop the gliding cells on the solid surface [16,17,19]. The molecule 105 nm long is shaped question mark and suggested for interaction with Gli349

Figure 1



Gliding machinery of *M. mobile*. **(a)** Gliding. Gradations of color, from purple to red, were applied to selected frames of 6 s of footage and integrated into one image [43]. The traces in the image show the gliding speed, the direction of gliding, and the time in relation to other traces. The gliding speed ranges from 2.0 to 4.5 μm per second. **(b)** Subcellular localization of proteins for gliding. Gli349 (MMOB1030, left) and F1 ATPase α subunit paralog (MMOB1660, right) are visualized by immunofluorescence and fluorescent protein fusion, respectively. **(c)** Rapid freeze-fracture deep-etch replica EM of cell. The outer leaflet of the lower cell membrane can be seen. A leg-like structure is marked by a yellow arrow. These images were adapted from the studies in [27,36•]. **(d)** Negative staining EM of internal 'jellyfish' structure. About 30 'tentacles' featured by particles about 20 nm in diameter are attached to 'bell' at the cell front. The bell is marked by an asterisk.

[16,17,19,25]. Therefore, this protein was assigned the role of 'crank'. Gli123, also essential for binding and gliding was assigned the role of 'mount,' which consists of providing the sites for protein localization, because the Gli349 and Gli521 proteins are distributed throughout the cell of a Gli123 nonsense mutant [16,19,20].

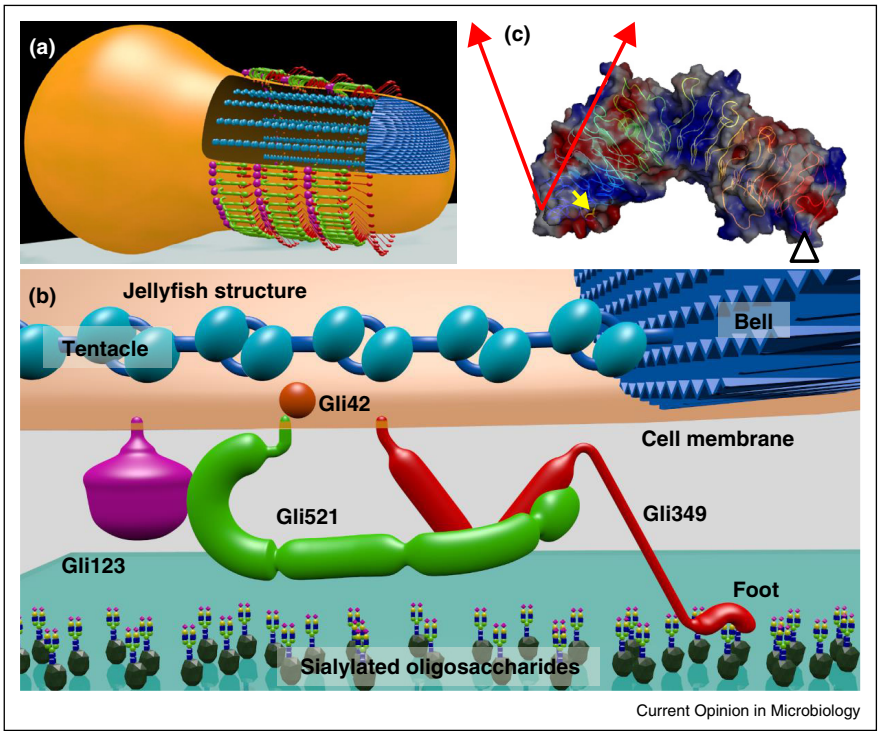
The details of the assembly of the gliding proteins on the membrane surface are still unclear, probably because the filamentous parts of Gli349 and Gli521 form a complex network [26]. Till date, the Gli349 molecules have been visualized on the cell surface by rapid freeze-fracture deep-etch replica electron microscopy (EM), one of the most effective EM methods in terms of image contrast and the imaging of fragile structures. The surface structures plausibly composed of Gli349 molecules have been shown to resemble string-like 'leg' appendages of approximately 50 nm in length sticking out from the membrane (Figure 1c) [27], which is consistent with the molecular shape of the isolated protein [23]. Integrating all the information reported to date [22–27], we suggest the putative surface assembly of gliding proteins shown in Figure 2b [6••,7••,8].

Binding and energy source

Both *M. mobile* and *M. pneumoniae* bind SOs, which are major structures on animal tissues and are the binding targets of many pathogenic factors [28•,29•,30]. Analyses using synthesized and purified SOs led to the conclusion that both mechanisms recognize SOs based on the detailed structures rather than only the negative charges. Measurement of the force needed to remove starved *M. mobile* cells from a solid surface suggested that the bond between Gli349 and SOs can be removed more easily forward than backward of cell (unpublished data), which may be involved in the gliding mechanism [7••,8,31••]. The binding site exists in the 'foot', that is, the C-terminal domain of Gli349 at the distal end of leg, because a single substitution from serine to leucine at position 2770 in this domain disabled the binding activity [16,19]. Although the foot domain composed of the 463 C-terminal amino acids does not share its sequence with other proteins, including receptors of SOs, the periodical appearance of the predicted β strand is shared in common with a structure — namely, the leucine-rich repeat represented by Toll-like receptors [32]. A predicted three-dimensional structure based on the secondary structures suggested a 'hook' shape containing the 2770th serine essential for binding at the N terminus of this domain (Figure 2c). This hook shape may cause the directed binding with the easy contortion of the foot, through a lever action around the C-terminal support point when the N-terminal end is pulled to the C-terminus side.

In general, information on direct energy sources is essential for the clarification of motility systems. In previous studies, therefore, we have tried to reactivate the so-called 'ghosts' of *M. mobile*, in which permeabilized dead

Figure 2



The gliding machinery. **(a)** A whole cell which can glide to the right. The gliding machinery appears as a membrane protrusion and is composed of surface and internal structures. The surface structure contains 450 molecules of each of Gli123 (pink), Gli349 (red), and Gli521 (green). The internal jellyfish structure (blue) is composed of a 'bell' and 'tentacles'. A similar structure was output by a 3D printer as shown in Supplementary material A. **(b)** Magnified image of a surface unit and part of the internal structure. The molecule of Gli349 (red), that is, the leg protein, resembles an eighth note and is anchored to the cell membrane at the N-terminal transmembrane segment [22–24]. From the N terminus, two short rigid arms, a long flexible part, and a C-terminal oval 'foot' are linked tandemly. Two short arms are linked by a foldable hinge. The foot has binding activity to the binding target, SOs [16,22]. Gli521 (green), a 'crank' protein, forms a trimer, showing a triskelion reminiscent of eukaryotic clathrin, through association at an end. The molecular shape of the Gli521 monomer consists of three parts connected by flexible hinges, an oval, a rod, and a hook from the N terminus. Gli521 is suggested to bind to Gli349 at the oval on the basis of analyses of mutants [16,34]. The bell of the jellyfish structure (blue), a solid structure, is located at the cell head and is filled with a hexagonal lattice of 12-nm periodicity. Dozens of tentacles are connected to the bell, and these tentacles are covered with particles 20 nm in diameter at intervals of approximately 30 nm. **(c)** Structure of the foot part of Gli349, modeled by Phyre2 [44], mainly based on a choline binding protein (PDB, 4cp6), with a sequence coverage of 95% and >90% confidence. The structure is similar to the leucine rich repeat represented by a Toll-like receptor, TLR4 [32], positioning the essential amino acid, the 2770th serine colored yellow at the site marked by a yellow arrow [16]. The N terminus at the left is connected to the other parts of Gli349. The foot may be removed more easily to the direction of the right arrow than the left one because of the lever action around the supporting point marked by an open triangle. The predicted PDB file is available as Supplementary material B.

cell bodies are reactivated for gliding. The addition of Triton X-100 to gliding *Mycoplasma* permeabilized the cells, causing the gliding motion to cease immediately. Subsequent addition of ATP reactivated these ghosts and caused them to glide, showing that the energy for *M. mobile* gliding is supplied by ATP hydrolysis and that detachment from the solid surface is an energy-dependent process [33–35]. Analyses of binding under depletion of ATP using gliding ghosts and inhibitors such as arsenate and azide have shown that energy input is essential for the rebinding of mycoplasmas in suspension (unpublished data). Anti-Gli521 antibodies stop the gliding and keep the cells on the glass but inhibit binding of the cells to solid surfaces, suggesting that the gliding machinery performs cyclic conformational changes, depending on the energy supply [17]. The ATPase reaction and the subsequent coupling to the interaction between foot and SOs should be the crucial information to understand this unique mechanism.

Internal structure

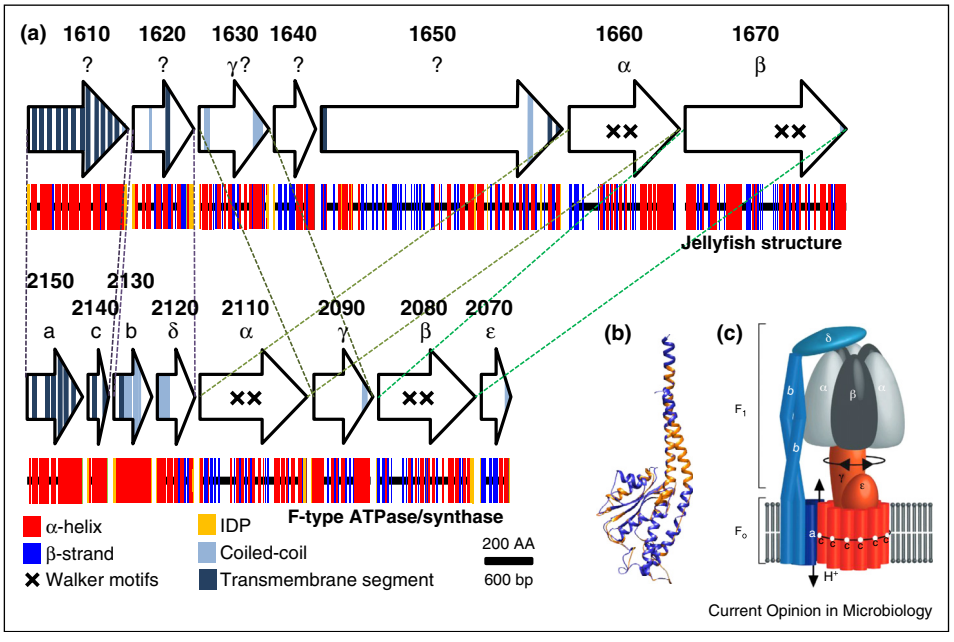
Removal of the cell membrane and cytosol by Triton extraction revealed a striking internal structure reminiscent of a jellyfish, which was composed of a bell and tentacles (Figures 1d and 2a,b) [26]. This structure contained at least seven proteins which are tandemly coded in a locus, suggesting that this locus is responsible for the main jellyfish structure [26,36•]. Interestingly, this locus

and that coding the surface gliding proteins occupy 83,163 and 66,243 bps, respectively, 10.7% and 8.5% in the reduced *M. mobile* genome composed of 777,079 bps, suggesting that the gliding motility is a determinant for their survival [7••,9]. Two proteins, coded by the ORFs, MMOBs 1660 and 1670, were highly similar to the α and β subunits of F-type ATPase/synthase, respectively. Although the other five proteins did not show obvious sequence similarity with other components of F-type ATPase/synthase, additional analyses suggested that there were, in fact, similarities in sequence features for these five proteins as well (Figure 3a,b). The prediction of the secondary structure and the modeling of the three-dimensional structures suggested similarities between MMOB1630 and MMOB2090, the γ subunit (Figure 3b). The a and c subunits of F-type ATPase/synthase are characterized by the frequent appearance of transmembrane segments, while the b and δ subunits feature transmembrane segments and coiled-coil parts [37,38]. These features and molecular sizes may suggest that the MMOBs 1610 and 1620 are derived through gene fusion, respectively from the 'a and c' and 'b and δ' subunits of F-type ATPase/synthase. The MMOB1610 protein is unlikely to rotate, because the counterparts of the rotor ring and ion channel-forming unit may be fused. The MMOB1650 protein, which includes three transmembrane segments, is also found in the fraction of the jellyfish structure. This protein may play a role in transmitting to the cell surface the movements generated by ATP hydrolysis in MMOBs 1660 and 1670, the α and β subunit paralogs.

Updated mechanism

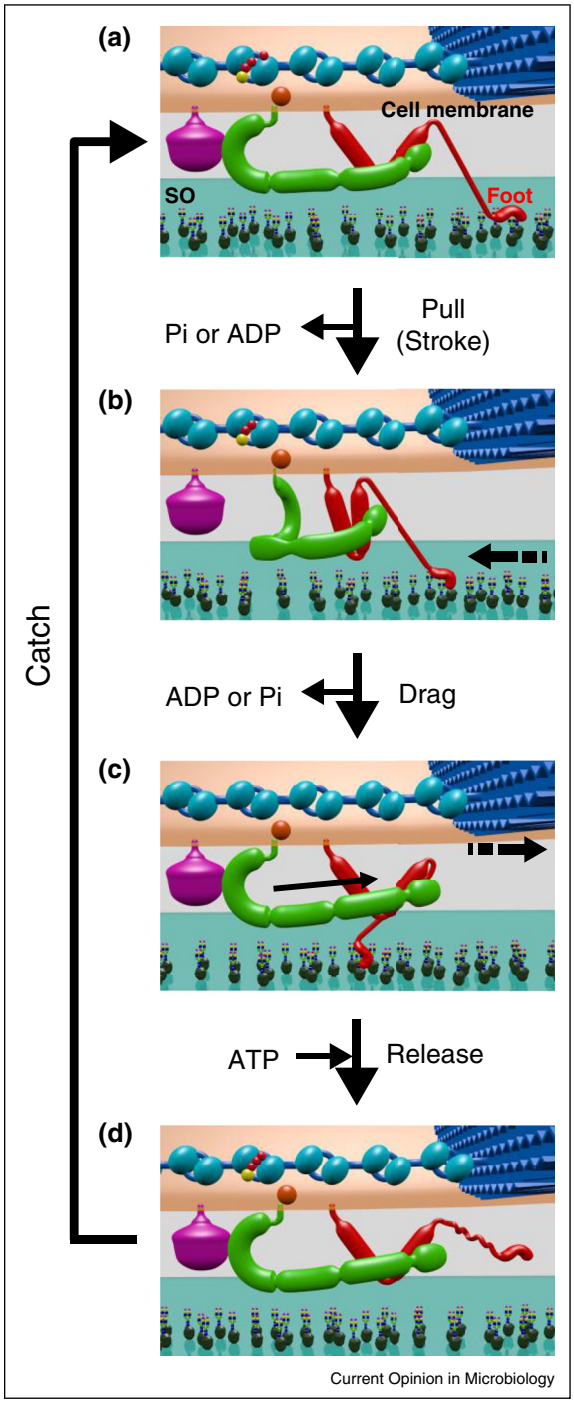
A working model called the centipede or power stroke model has been proposed in which cells are propelled by legs composed of Gli349 proteins repeatedly binding to and releasing from the glass [7••,8,31••]. Each gliding unit participates in a mechanical cycling through the following states (Figure 4) and transition steps: catch, pull (stroke), drag, and release. The pulling steps appeared to consist of individual 70-nm steps when the cell displacement was analyzed under limited binding by the addition of free SOs [33,39•]. These steps should be caused by ATP hydrolysis at the complex of MMOBs 1660 and 1670. Then, how is the ATPase cycle coupled with these steps? A new ATP molecule should be necessary for release, because the ghosts stop gliding on the glass surface when ATP is depleted by permeabilization

Figure 3



Comparison between component proteins of the jellyfish structure proteins and F-type ATPase/synthase. **(a)** Comparison of ORFs in the *M. mobile* genome. The jellyfish structure (upper) and F-type ATPase/synthase (lower) from *M. mobile* are shown with gene IDs, sequence features, and annotations for F-type ATPase [26,40•]. The related or possibly related genes are connected by broken lines. Prediction of the two-dimensional structures by PSI-PRED suggested similarities between MMOBs1630 and 2090. **(b)** Overlaid structures of MMOBs 2090 (blue) and 1630 (gold), both of which were modeled by Phyre2 [44], based on the structure of an F-type ATPase gamma subunit from *E. coli* (PDB, 3oaa), with sequence coverages of 97% and 75%, and 100% and >97% confidences, respectively. The predicted PDB file is available as Supplementary materials C and D. **(c)** General structure of a bacterial F-type ATPase/synthase composed of nucleotide binding stator subunits, 'α' and 'β'; a central stalk, 'γ'; a rotor ring, 'c'; an ion channel forming subunit, 'a'; a peripheral stalk, 'b' and 'δ'; and a small central stalk subunit, 'γ'. This figure was adapted from the study in [37].

Figure 4



Updated centipede model for *M. mobile* gliding. Refer to Figure 2b for the detailed structures. This model is also available as Supplementary material E. **(a)** The leg (Gli349) catches an SO on the solid surface. The tension applied to the leg from the front triggers a conformational change in Gli349 by movements transmitted from the internal motor, causing the pull (stroke), using the energy coupled with the conversion of ATPase forms. **(b)** Cell movements occurring as a result of other legs pull the units forward, inducing conformational changes. **(c)** Continuous pulling in the forward direction removes the foot from the SO, coupled with the incorporation of new ATP. The foot can be removed preferentially in the forward direction, and this directed binding property causes directed movement with a directed stroke. **(d)** The detached foot catches an SO again at the proper position, after the conversion of ATPase intermediates.

[34]. Because the leg conformation should change before the foot catches a new SO, the unit should be in a form, perhaps an ADP + Pi form, other than the ATP form. The stroke is coupled with release of ADP or Pi, because this step should require a large amount of energy.

Evolutional origin

As discussed above, the gliding of *M. mobile* has been suggested to have arisen through a hybridization of an adhesin and a rotary ATPase. If so, however, what was the specific origin of this gliding? The genome analysis revealed that another set of paralogs of *M. mobile* jellyfish proteins is widespread in many *Mollicute* genomes, including *M. mobile* and nongliding species, suggesting a possible scenario for the evolution of gliding [40•]. *Mollicutes* evolved from a low-GC branch of Gram-positive bacteria through a genome reduction in parasitic life cycles [1]. They lost the peptidoglycan layer and instead covered their surfaces with special proteins that were responsible for evading the host immune system and binding to the hosts [4,41,42]. Separately, the gene of F-type ATPase/synthase was duplicated, evolved to play a role different from ion transport, and became widespread in *Mollicute* species [40•]. This new type ATPase/synthase rotates on the membrane and accidentally touched and drove the neighboring adhesin molecules. The movement of adhesin would have resulted in gliding motility and conferred a major advantage for spreading on the host and evading the immune system.

Conclusions

M. mobile gliding has been studied since 1997, and recent progress in this field suggests that all outstanding questions on the components, architecture, mechanisms, and evolutional changes related to this activity may be answered in the next decade. However, the mechanisms of two other motility systems in *Mollicutes* are probably to require more time to decipher.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mib.2015.08.010.

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- •• of outstanding interest

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